New Microcrystal Tests for Controlled Drugs, Diverted Pharmaceuticals, and Bath Salts (Synthetic Cathinones)

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BACKGROUND

Traditional light microscopy and microcrystal tests have been used together for more than 100 years. They have proven useful when automated instrumental analysis is unavailable or impractical; for example, if mixtures of one or more drugs, excipients, diluents or adulterants are present, or when the drug is held in alternative delivery devices such as gels or transdermal patches. Furthermore, while some crime laboratories may lack certain automated instrumental capabilities, most have light microscopes and properly trained microscopists.

Microcrystal tests using polarized light microscopy (PLM) can identify most illicit drugs, diverted pharmaceuticals, and synthetic cathinones (psychoactive bath salts) specifically and quickly (usually within a few minutes) and are inexpensive compared to other methods. In addition, proper use of the light microscope and microcrystal tests can check and confirm the results obtained by alternative methods. It should be explicitly noted, that good scientific practice requires the use of a positive and negative control that should be implemented with the use of microcrystal tests. This ensures that the reagents are functioning properly and that the analyst can recognize the crystal morphologies and optical properties that indicate a positive result, as well as the ability to recognize crystal morphologies and optical properties that do not indicate a positive result. The original compendium of microcrystal tests, together with the addition of these recently discovered microcrystal tests for 9 new drugs, will continue to fulfill a critical need for reliable analytical methods and assist forensic scientists and other researchers in their work.

MICROCRYSTAL TESTS

A Modern Compendium of Microcrystal Tests for Illicit Drugs and Diverted Pharmaceuticals includes 19 drugs for which microcrystal tests using various reagents have been previously developed. It is used today by forensic scientists in the crime laboratory and by researchers in the analytical chemistry laboratory. *New Microcrystal Tests for Controlled Drugs, Diverted Pharmaceuticals, and Bath Salts (Synthetic Cathinones)*, contains research on 9 additional drugs, including some psychoactive bath salts, for which microcrystal tests had not previously been discovered or developed. Together, the compendiums describe in detail the microcrystals formed for 28 drugs with various reagents for each test and include photomicrographs, morphology illustrations, optical properties, notes, and infrared (IR) spectra of the resultant microcrystals.

Most drugs in the original compendium include two or three reagents that may be used for their identification; in a few cases, only one reagent is provided. Reagents for the new research were first determined as candidates based on their availability, use, and success in microcrystal tests for related chemical compounds. The methods were derived from the technical literature with known compounds of interest and were subjected to rigorous testing. Once a reagent was found to form characteristic microcrystals, the reagent and test method were thoroughly evaluated. As a result, each reference listed in the new monographs for the 9 additional drugs is directly related to the reagent formula and a known documented alternative use for the reagent. For each of the 9 additional drugs,

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the authors include two reagents, which were found to be reliable, accessible, and practical.

Techniques have also been developed for the additional drugs with common adulterants that may inhibit or distort crystal formation, including butylone, BZP, caffeine, ethylone, lidocaine HCl, MDPV, 4-MEC, mephedrone, methylone, alpha-PVP, and TFMPP. All procedures were vetted and evaluated by McCrone Research Institute research microscopists, together with practicing forensic scientists in other collaborative laboratories. The compendium and monographs include recommended protocols, reagents, morphology of crystals (with numerous photomicrographs), IR spectra of microcrystals, and potential interferences. In addition, they include optical and crystallographic properties of the microcrystals.

Each monograph includes the following topics for each drug: reagents; test methods; sensitivity of the test and limit of detection; time required for crystal formation; crystal morphology; evaluation of the tests in the presence of common excipients, diluents, and adulterants (for street drug samples) or combination drugs (for pharmaceutical preparations); and evaluation of the tests for drugs from selected pharmaceutical delivery devices, e.g. tablets, capsules, gels, transdermal patches, and oral solutions when applicable.

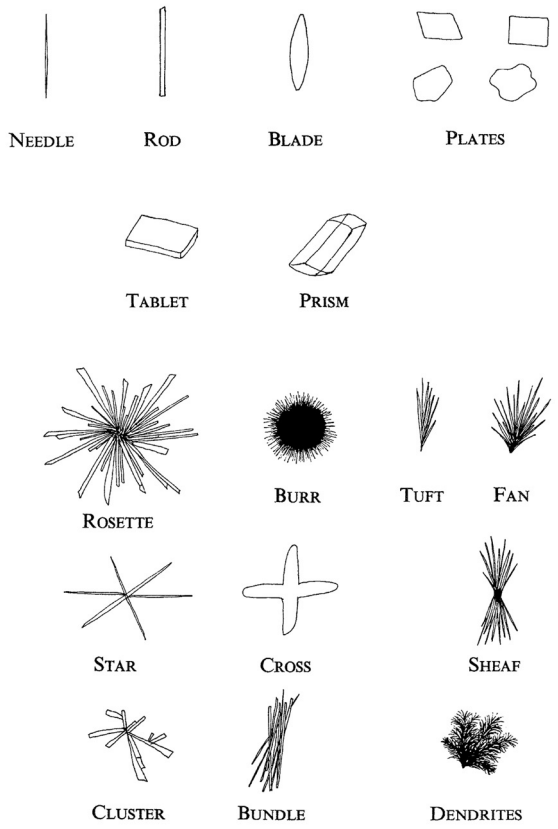

Figure 1. Microcrystal morphology (from Clarke; used with permission).

Limit of Detection

The limit of detection (LOD), or minimum amount of sample required to obtain a positive result, i.e. typical crystal formation, was determined for each drug and reagent in the compendium. Some previous researchers referred to using samples "the size of a period on a printed page." The amount suggests a minimum required sample quantity and provides a means to compare the sensitivity of all the microcrystal tests. An analogous unit of measurement was established for this compendium wherein sample size was measured in units of "PPP," a quantity with an approximate diameter the size of a single period on a printed page. This unit represents a quantity of sample that fills the area of a period printed or displayed at 100% in Times New Roman 10-point font. The weight of 1 PPP is approximately 0.1 mg. All microcrystal tests in the compendium specify a LOD (usually 1 PPP) for each drug and reagent; however, the LOD is a lower limit, and more material can be tested with similar results. Pharmaceutical products included in the compendium were tested at various dosages or concentrations, and in most cases, the lowest dosage pharmaceutical and the lowest quantity of material required for a successful test was specified.

Crystal Morphology

Descriptions of typical crystal morphology were often used in reference to Clarke (Figure 1). There are some cases where Clarke's general descriptions are used with additional terms for microcrystals that resemble easily recognizable objects: parallelograms, nails, bow ties, coffins, dahlia flowers, pants, wrapped candy, etc.

Pharmaceuticals, Adulterants, Other Drug Interactions, and Alternative Delivery Devices

The compendium and monographs include commonly encountered adulterants and excipients that were tested in several ratios with the drugs (5:1, 1:1, and 1:5) to determine the success of each microcrystal test and reagent. In most cases, the microcrystal test was successful and the drug was detected in these ratios. However, in a few cases, the drug produced no crystals, was not reproducible, or did not produce typical crystals in the presence of the adulterant or excipient. Some pharmaceuticals included several different drugs or ingredients, and the additional drugs interfered with the microcrystal test, or the drug was present in such low concentration that

typical crystals were distorted or did not form. In these instances, micro-scale extractions were performed in order to extract, isolate, or concentrate the drug. The extractions carried out using microcentrifuge tubes take only a few minutes and are described in the compendium.

In addition to tablets and capsules, several pharmaceuticals employing alternative delivery devices (e.g. oral solutions, extended-release formulations, gels, and transdermal patches) were tested in order to determine the success or failure of the microcrystal tests. In some cases, the microcrystal test produced no positive results directly, and micro-scale extractions were required. After some modifications to the test methods, many microcrystal tests were successful on alternative delivery devices and are described in the compendium.

Fourier Transform Infrared Microspectroscopy

Infrared spectra of the microcrystals obtained by Fourier transform IR microspectroscopy were not available in any of the references and are now included in this compendium. It was observed that the spectra obtained from the drugs are different than the spectra obtained from typical microcrystals of the same drug. There are sometimes differences in peaks and small shifts in peak positions, and there are differences that may occur in the spectra of microcrystals for the same drug when using different reagents.

IR spectra files (.spc and .jpg formats) for microcrystal tests are available for download on the McCrone Research Institute website, www.mccroneinstitute.org.

PLM OPTICAL PROPERTIES

Refractive Indices

The refractive indices of some microcrystals were difficult to determine because they must be dried, not obscured by recrystallized reagent, and excess liquid must be wicked away before applying the refractive index liquids. Crystals in ordinary aqueous reagents were most easily dried at room temperature, while those in acidic reagents needed to be washed with a solvent such as ethanol or chloroform and then dried. The following is the procedure used for washing the crystals: Excess reagent was wicked with a lab tissue or filter paper. A drop of solvent was placed on a slide near the typical crystals that formed, and then a tungsten needle was used to wick up some of the solvent to draw it over the crystals and wash free the reagent from the crystals. This may require multiple attempts to sufficiently remove the reagent. Some of the microcrystals have refractive indices greater than 1.700, which are considered very high and are above the limit at which many laboratories are capable of determining with readily available refractive index liquids. When microcrystals exhibited a refractive index greater than 1.700, this result was recorded, and exact values were not pursued further.

Estimated Birefringence

Birefringence (B) was determined by measuring the thickness of the crystal using a calibrated ocular scale and estimating the interference colors observed in crossed polars with PLM. The birefringence was then calculated using a Michel-Lévy interference color chart or the classic birefringence equation, $B = R / (1000 \times T)$, where R is retardation (interference color value, in nanometers) and T is crystal thickness (in micrometers). Birefringence for the typical microcrystals was estimated to be low when the values were less than 0.010, moderate when they were between 0.010 and 0.050, or high when they were greater than 0.050.

Sign of Elongation

The sign of elongation was determined for microcrystals that are elongated. If the refractive index parallel to the long axis (length) is greater than the refractive index perpendicular to the long axis (width), then the crystal has a positive sign of elongation. If the opposite is true, it has a negative sign of elongation.

Interference Figures

Interference figures were difficult to obtain on the microcrystals. Many of the microcrystals were not a suitable shape or size or were not properly oriented to observe an interference figure. However, there are a few crystals in the compendium that did show good interference figures. When an interference figure was observed, its uniaxial or biaxial character was recorded together with the optic sign.

METHODS AND TECHNICAL NOTES

The procedures used throughout the research and presented in the compendium and monographs are standard procedures employed by most microscopy laboratories and will be familiar to any microscopist. Techniques that may be less common are explained in the appropriate section for each drug. However, there are technical details about the tests throughout the compendium that should be noted: most microcrystal tests are performed in an exposed reagent drop without using a coverslip. Unless specified, a coverslip was not used in performing these tests. Occasionally, a coverslip was placed on the reaction drop after crystal growth occurred in order to obtain better quality photomicrographs.

Most of the microcrystal tests required less drug material and, therefore, less liquid reagent than traditional laboratory dropper bottles provide. A micropipette was used to obtain smaller quantities of liquid. If a micropipette is not available, tiny drops of solvent or reagent can be made by using a tapered glass rod. A tapered glass rod is made from a length of cylindrical glass approximately 10 cm in length and 2–3 mm in diameter that has been drawn out in a flame to about 1 mm diameter at the tip, then polished to a flat, blunt end. The glass rod can be used to obtain small drops of solvent or reagent by simply teasing a drop from a bottle dropper. The bottle dropper is squeezed slightly, allowing a small amount of liquid to exit the tip as the glass rod is drawn across the opening. This creates a micro-drop, approximately 5 µL, on the tip of the glass rod. The drop can then be placed on a glass slide or coverslip in preparation for the microcrystal test. A 5 µL drop, after being placed on the glass slide or coverslip, will be about 5 mm in diameter.

Glass rings used during the volatility tests have the following specifications: 17 mm outer diameter, 14 mm inner diameter, 1 mm wall thickness, and 5 mm height. Different diameter rings and glass concavity slides should give similar results, however, the microscope may have difficulty focusing with glass rings that are more than 5 mm in height, especially when using high-magnification objectives. Glass is the preferred material for the rings because it is inert, however, other materials may be substituted if they will not interfere with the microcrystal tests.

Reagent formulations are written using the quantities given in the original sources but can be halved, quartered, or otherwise adjusted as needed. Unless otherwise noted, the reagents are stable for years, if stored properly. If the age or condition of a reagent is uncertain, the test should be performed on a known drug sample to ensure the reagent is working properly.

Data, including photomicrographs, were obtained using research-grade drug standards in order to acquire the highest quality results. Pharmaceuticals and street drug samples tested with the reagents typically yielded the same microcrystals. However, in some rare cases, certain combination drugs or adulterants may have caused the test to be unsuccessful. These instances are noted in the appropriate drug and reagent sections, together with any alternative test methods.

Pharmaceutical tablets are often coated or encapsulated with inert ingredients that do not contain any drug material. Therefore, when sampling from a pharmaceutical tablet, the tablet was first broken in order to expose the inner portion. A needle or sharp instrument was then used to break off small pieces from the center, without the coating. The drug material is sometimes present as colorless particles, which can be distinguished from other fillers and binders (e.g. microcrystalline cellulose, starch, etc.), when viewed with a stereomicroscope. The drug particles may be euhedral (well-formed), causing them to appear shiny in reflected light. When these crystals are present, they should be selected and removed individually for the microcrystal tests.

CONCLUSION

A Modern Compendium of Microcrystal Tests for Illicit Drugs and Diverted Pharmaceuticals is presented in a PDF file and comprises 19 drugs. It includes reagents, microcrystal test methods, optical properties, and IR spectra. *New Microcrystal Tests for Controlled Drugs, Diverted Pharmaceuticals, and Bath Salts (Synthetic Cathinones)*, contains 9 additional drugs, including psychoactive bath salts, for which microcrystal tests had not previously been discovered or developed. Both publications will be updated with additional drugs, reagents, and microcrystal tests when such data become available.

AUTHOR CONTRIBUTIONS

Sebastian Sparenga and Meggan King performed and evaluated the microcrystal tests, documented the optical properties, and assisted in the format, layout, and design of the monographs. Sparenga performed the IR micro-

spectroscopy. Dean Golemis designed the layout, created the pages, and edited the content. Gary Laughlin provided the editorial and technical review and overall project management. All authors read and approved the final documents.

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